Responses of *Nasonovia ribisnigri* (Homoptera: Aphididae) to Susceptible and Resistant Lettuce

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ABSTRACT Nymphs and alates of aphid *Nasonovia ribisnigri* (Mosley) (Homoptera: Aphididae) were tested on 10 lettuce cultivars with *N. ribisnigri* resistance gene *Nr* and 18 cultivars without the resistance gene in various bioassays. Bioassays used whole plants, leaf discs, or leaf cages to determine susceptibility of commercial lettuce cultivars to *N. ribisnigri* infestation and to evaluate screening methods for breeding lettuce resistance to *N. ribisnigri*. Resistant and susceptible plants were separated in 3 d when using whole plant bioassays. Long-term ($\geq$7 d) no-choice tests using leaf cages or whole plants resulted in no survival of *N. ribisnigri* on resistant plants, indicating great promise of the *Nr* gene for management of *N. ribisnigri*. Effective screening was achieved in both no-choice tests where resistant or susceptible intact plants were tested separately in groups or individually and in choice tests where susceptible and resistant plants were intermixed. Leaf discs bioassays were not suitable for resistance screening. All lettuce cultivars without the resistance gene were suitable hosts for *N. ribisnigri*, indicating the great importance of this pest to lettuce production and the urgency in developing resistant lettuce cultivars to manage *N. ribisnigri*.

KEY WORDS plant resistance, resistance screen, cultivar, breeding, bioassay

The aphid *Nasonovia ribisnigri* (Mosley) (Homoptera: Aphididae) has been a major pest of lettuce in western Europe and Canada for many years (van Helden et al. 1993, Martin et al. 1995, Rufingier et al. 1997, Ryder 1998). *N. ribisnigri* also occurs in the eastern and western United States. (Ryder 1998) and has become a major pest of lettuce in the Salinas Valley, CA, since its detection in 1998 (Chaney 1999). *N. ribisnigri* prefers to feed in the hearts of lettuce plants and is difficult to control with contact insecticides, especially on head lettuce. Lettuce contaminated with *N. ribisnigri* is unsalable. *N. ribisnigri* is also a vector of virus diseases, including gooseberry veinbanding virus, cauliflower mosaic virus, cucumber virus, and lettuce mosaic virus (Davis et al. 1997), although its ability to transmit lettuce mosaic virus in California has not been reported.

In Europe, chemical control of *N. ribisnigri* has produced resistance to a variety of insecticides (Barber et al. 1999, Rufingier et al. 1997). Breeding for resistant lettuce varieties yielded resistant lettuce lines, including 'Dynamite' (van der Arend et al. 1999). The resistance to *N. ribisnigri* is conferred by a single incompletely dominant gene, *Nr* that was transferred from *Lactuca virosa* L. to cultivated lettuce *L. sativa* L. by using *Lactuca serriola* L. as a bridge species (Eenink et al. 1982, Ryder 1998). Lettuce possessing *Nr* also showed certain degrees of resistance to green peach aphid, *Myzus persicae* (Sulzer) (Reinink et al. 1988, Montllor and Tjallingii 1989).

N. ribisnigri responds differently to resistant and susceptible lettuce plants (Montllor and Tjallingii 1989, van Helden 1990). *N. ribisnigri* makes more but shorter probes on resistant plants than on susceptible plants (Montllor and Tjallingii 1989). Alates of *N. ribisnigri* inoculated on resistant lettuce plants more likely leave the resistant plants than alates on susceptible plants (van Helden et al. 1992). Transfer of *N. ribisnigri* after 2 d on resistant plants to susceptible plants showed no sign of intoxication (van Helden et al. 1993). *N. ribisnigri* also was found to prefer phloem sap from susceptible plants over that from resistant plants, suggesting that resistance is likely based on a feeding deterrent activity of the phloem sap in the resistant plants (van Helden et al. 1995). Confining *N. ribisnigri* to resistant plants of near isogenic lines resulted in higher mortality, unsuccessful development to adulthood, and no reproduction compared with *N. ribisnigri* on susceptible plants (van Helden et al. 1993).

In the United States, studies on *N. ribisnigri* are very limited because of the short history of *N. ribisnigri* as a major pest (Chaney 1999; Palumbo 2000, 2003; Palumbo and Hannan 2002; Liu 2004). Information on susceptibility of local commercial lettuce varieties to *N. ribisnigri* and how *N. ribisnigri* strain in the United States responds to resistant lines of lettuce are lacking. In the current study, we tested both nymphs and alates of *N. ribisnigri* in various types of bioassays by using whole plants, leaf discs, and leaf cages with commercial lettuce cultivars and breeding lines with the resistance gene *Nr.* The objectives of the study were to evaluate different bioassay methods for screening resistance to *N. ribisnigri* in lettuce breeding based on insect survival and reproduction and to gain a better understanding of responses of *N. ribisnigri* to resistant and susceptible lettuce cultivars and susceptibility of commercial lettuce cultivars to the pest.

Materials and Methods

Lettuce Plants. Lettuce plants from 10 breeding lines with *Nr* gene for resistance to *N. ribisnigri* and 18 cultivars without the resistance gene *Nr* were used. They belong to five lettuce types: butterhead, crisphead, leaf, lolo rosa, and romaine. For brevity, breeding lines also were referred as cultivars. The 10 cultivars with the resistance gene *Nr* were '83-67-RZ', '85-45 RZ', 'Barcelona', 'Campionas', 'Dynamite', 'Elenas', 'Fortunas', 'Irina', 'Krinas', and 'Vetonas'. The 18 lettuce cultivars without the resistance gene were 'Bibb', 'Big Red', 'Calmar', 'Dark Green Boston', 'Darkland', 'Lobjoits', 'Lollo Rossa', 'Margarita', 'Oakleaf', 'Pacific', 'Parris Island', 'Prizehead', 'Salinas', 'Shining Star', 'Tiber', 'Two Star', 'Valmaine', and 'Vanguard 75′. The resistant cultivars were originally obtained from Rijk Zwaan (Salinas, CA). van der Arend et al. (1999) detailed the breeding of Dynamite. All breeding lines and cultivars used in this study were obtained from the USDA–ARS lettuce germplasm collection maintained at Salinas, CA.

Lettuce plants were grown in potting soil in 7.6- by 7.6-cm pots. Lettuce seeds (one to two seeds per pot) were placed on the top of wetted potting soil in the pots and covered with a thin layer of fine sand. Plants were watered daily. Plants were fertilized with slow release fertilizer (24-4-8, N–P–K) after 2 wk and fertilized with foliar spray fertilizer formulation (36-6-6) weekly thereafter. After germination, plants were grown for ≥1 mo before they were used for bioassays.

Insects. *N. ribisnigri* was collected from the Spence field in Salinas in 2001 and reared on Parris Island and Salinas lettuce in large screened cages in a greenhouse. Alates and <24-h-old nymphs were used for bioassays. Alates were taken randomly from the colony for bioassays. Nymphs were obtained by confining ≈20–30 alates on a small lettuce plant in a cage (11.5 cm in diameter by 13 cm in height) modified from a clear plastic cup with screened windows on the wall and the top and harvesting offspring within 24 h. All insect rearing and bioassays were conducted in the greenhouse.

Survival of Nymphs on Plants. Survival of *N. ribisnigri* nymphs on plants of susceptible and resistant lettuce cultivars was evaluated in a greenhouse. Ten nymphs (<24 h) were placed on each lettuce plant. In the first experiment, lettuce cultivars used were Barcelona, Dark Green Boston, Dynamite, Margarita, Calmar, Salinas, Lobjoits, Parris Island, and Valmaine. Infested plants were placed in water-filled trays in large screen cages on a greenhouse bench and spaced to prevent touching of leaves between adjacent plants. Numbers of nymphs on each plant were recorded at 3 and 5 d. In each bioassay, two plants from each cultivar were used. The bioassay was replicated three times. In total, 54 lettuce plants were infested.

Survival and Reproduction of Alates on Plants. Susceptible lettuce Dark Green Boston, Margarita, Calmar, Salinas, Lobjoits, Parris Island, and Valmaine and resistant Dynamite and Barcelona were used to evaluate survival and reproduction of *N. ribisnigri* alates on plants. Alates were randomly collected from the aphid colonies and placed on each plant (10 alates per plant). Each infested plant was covered with a cup cage as detailed above and placed in a water-filled tray. Surviving alates were counted at 3 and 5 d. Numbers of live and dead nymphs also were counted at 5 d. Two plants from each cultivar were tested in each bioassay. The bioassay was replicated three times. In total, 54 lettuce plants were infested with 540 alates.

Host Preference and Reproduction of Alates in Choice Bioassay. Susceptible lettuce Dark Green Boston, Margarita, Calmar, Salinas, Lobjoits, Parris Island, and Valmaine and two resistant Dynamite and Barcelona were grown in pots for ≈2 mo and were arranged randomly in 61- by 61- by 61-cm screen cages in a greenhouse. Plants were set in petri dishes to provide water reservoirs. Two plants from each cultivar were placed in one cage. Alates (>100) were collected in a large plastic cup from the colonies. The cup with alates was then suspended at the top center of the cage to allow alates to disperse from the cup. Live and dead alates on each plant were counted daily for five consecutive days after the release of alates, and plants were rerandomized in the cage after counts were made each day. Numbers of live and dead nymphs on each plant were counted at 5 d. The test was replicated three times. In total, 54 plants were included, and >400 alates were released in the three replications.

Survival and Population Growth on Susceptible and Resistant Cultivars in Separate and Mixed Treatments. Eight resistant cultivars (83-67-RZ, Campionas, Dynamite, Elenas, Fortunas, Irina, Krinas, and Vetonas) and 13 cultivars without the resistant allele (Bibb, Big Red, Darkland, Lollo Rossa, Margarita, Pacific, Parris Island, Prizehead, Salinas, Shining Star, Tiber, Two Star, and Vanguard 75) were tested in 61- by 61- by 61-cm screen cages. Plants were first inoculated with 10 (1–2-d-old) nymphs. Then, the susceptible plants and resistant plants were grown in separate cages (separate treatment) and in the same cage (mixed treatment). Numbers of aphids on each plant were recorded at 14 and 21 d.

In the separate treatment, one plant from each of susceptible cultivars was inoculated with *N. ribisnigri* nymphs and placed in one cage, and one plant from each of the resistant cultivars was inoculated with *N. ribisnigri* nymphs and placed in a separate cage. Five cages (replications) were used for susceptible cultivars and five cages were used for the resistant cultivars. In the mixed treatment, one plant from each of susceptible (except Vanguard 75) and resistant cultivars were arranged randomly in one cage after they were inoculated with *N. ribisnigri* nymphs. Three cages (replications) were used for the mixed treatment. In total, 165 plants from all 21 cultivars were used in the experiment.

Survival and Reproduction of Alates on Leaf Discs. Discs (12 mm in diameter) were taken from leaves of lettuce plants of 14 susceptible cultivars (Bibb, Big Red, Darkland, Lollo Rossa, Margarita, Oakleaf, Pacific, Parris Island, Prizehead, Salinas, Shining Star, Tiber, Two Star, and Vanguard 75) and 10 cultivars with the resistance gene *Nr* (83-67-RZ, 85-45-RZ, Barcelona, Campionas, Dynamite, Elenas, Fortunas, Irina, Krinas, and Vetonas). Leaf discs from each cultivar were placed on filter paper discs placed on top of 5% agar in separate petri dishes (10 cm in diameter). One leaf disc was placed in each petri dish. Five alates randomly taken from the insect colonies were placed on each leaf disc. Petri dishes with leaf discs and aphids were placed in an environmental chamber at 21°C and a photoperiod of 12:12 (L:D) h. Surviving alates and offspring were counted at 3 d. The test was replicated two times.

Survival and Reproduction of Alates in Leaf Cages. Clip-on leaf cages were placed on leaves of susceptible Oakleaf and resistant 85-45-RZ in a greenhouse. The cages were made from ≈8 mm sections of soft vinyl tubing (8 mm i.d.) and long hairpins. The tops of cages were sealed with screen. Cages were placed so that the alates were exposed to the abaxial leaf surface. One *N. ribisnigri* alate was placed in each cage. Live alates and offspring were counted at 7 d. Two tests were conducted. Total numbers of 20 cages and 20 *N. ribisnigri* alates were tested on the susceptible and resistant lettuce plants, respectively.

Data Analyses. Data were analyzed using the Fit model platform of JMP statistical software (SAS Institute 2002). Data on survival rates were transformed by arcsine$\sqrt{x}$, and data on insect counts were transformed by $\sqrt{x}$ before analysis to meet the assumptions of normality and homogeneity of variance. Tukey–Kramer multiple comparisons were used to separate multiple means. Analysis of variance (ANOVA) and multiple range tests were conducted on survival rates of nymphs and alates, number of offspring, and offspring survival rate.

For the experiment on host preference and reproduction of alates in choice bioassay, the alate counts of each day were analyzed separately to determine distribution pattern of alates among different cultivars. Offspring at day 5 included both live and dead nymphs and were analyzed for offspring production and survival on different cultivars. For the experiment on effects of separation and mixing of susceptible and resistant plants, all cages (replications) were pooled in data analysis to increase statistical power. Cultivars were considered as nested within plant type. The separate treatment and the mixed treatment were analyzed together and separately to determine treatment effects and to determine aphid distributions among cultivars within each treatment. For the experiment on alate survival and reproduction on leaf discs, data from all cultivars within susceptible and resistant plant types were pooled separately to increase statistical power in the ANOVA.

Table 1. Mean ± SE percentage of survival of *N. ribisnigri* nymphs at 3 and 5 d on nine lettuce cultivars

Cultivar	Nymphal survival (%)	
	3 d	5 d
Dark Green Boston	83.3 ± 5.6a	70.0 ± 9.3a
Dynamite	21.7 ± 4.8b	1.7 ± 1.7b
Margarita	78.3 ± 7.5a	71.7 ± 8.7a
Barcelona	18.3 ± 5.4b	10.0 ± 6.8b
Calmar	80.0 ± 4.5a	70.0 ± 4.5a
Salinas	88.3 ± 3.1a	88.3 ± 3.1a
Lobjoits	81.7 ± 3.1a	78.3 ± 1.7a
Parris Island	68.3 ± 9.5a	68.3 ± 9.5a
Valmaine	83.3 ± 3.3a	61.7 ± 10.5a

For each cultivar, $n = 60$. Survival data were transformed by arcsin$\sqrt{x}$ before analysis of variance. Values in each column followed by different letters were significantly different (Tukey–Kramer multiple range test; $P \leq 0.05$ [SAS Institute 2002]).

Results

Survival of Nymphs on Plants. There were significant differences in survival rate of *N. ribisnigri* nymphs on susceptible and resistant lettuce cultivars at 3 and 5 d ($F = 13.69$; df = 8, 43; $P < 0.0001$ for 3 d; $F = 20.08$; df = 8, 43; $P < 0.0001$ for 5 d) (Table 1). For susceptible cultivars, the survival rate of aphids at 3 d ranged from 68.3 to 88.3% and showed no decline or slight decline from 3 to 5 d. In contrast, the survival rates of *N. ribisnigri* on resistant Dynamite and Barcelona at 3 d were only 21.7 and 18.3%, respectively, and declined to 1.7 and 10.0% at 5 d. There were significant differences in nymphal survival between the susceptible cultivars and resistant cultivars at 3 and 5 d (Table 1).

Survival and Reproduction of Alates on Plants. There were significant differences in survival rate of *N. ribisnigri* alates on susceptible and resistant lettuce cultivars at 3 and 5 d ($F = 13.91$; df = 8, 43; $P < 0.0001$ for 3 d; $F = 21.05$; df = 8, 43; $P < 0.0001$ for 5 d) (Table 2). The lowest survival rates on susceptible cultivars were 81.7% at 3 d and 70% at 5 d. On the resistant cultivars, however, the highest survival rates of alates were 38% at 3 d and 15% at 5 d. The survival rates on the resistant lettuce were significantly lower than those on susceptible cultivars both at 3 and 5 d (Table 2).

Aphid offspring production and offspring survival also differed significantly among cultivars ($F = 31.52$; df = 8, 43; $P < 0.0001$ for offspring production; $F = 12.87$; df = 8, 43; $P < 0.0001$ for offspring survival) (Table 2). On susceptible cultivars, total numbers of offspring produced on each plant at 5 d ranged from 39.8 to 51.2, and there were no significant differences among them. Alates on the resistant cultivars, however, had significantly lower production of offspring. Offspring survival rate also varied significantly, with 65.5 and 36.5%, respectively, on Dynamite and Barcelona plants and >94% on all susceptible cultivars (Table 2).

Table 2. Means ± SE for percentage of survival, offspring production, and offspring mortality of *N. ribisnigri* alates on nine lettuce cultivars

Cultivar	Alate survival (%)		Total no. of offspring	Offspring survival (%)
	3 d	5 d		
Dark Green Boston	81.7 ± 3.1a	70.0 ± 5.2a	41.0 ± 4.2a	96.7 ± 1.2a
Dynamite	35.0 ± 13.8b	8.3 ± 6.5b	6.0 ± 1.5b	34.5 ± 16.0c
Margarita	91.7 ± 3.1a	78.3 ± 4.8a	48.5 ± 5.6a	97.2 ± 1.5a
Barcelona	38.3 ± 14.5b	15.0 ± 7.6b	13.3 ± 4.6b	63.5 ± 9.3b
Calmar	91.7 ± 4.0a	80.0 ± 8.2a	51.7 ± 5.1a	94.3 ± 1.9ab
Salinas	98.3 ± 1.7a	86.7 ± 6.2a	52.2 ± 8.2a	97.8 ± 1.4a
Lobjoits	90.0 ± 4.5a	83.3 ± 3.3a	53.0 ± 6.2a	96.8 ± 1.3a
Parris Island	95.0 ± 3.4a	83.3 ± 9.9a	46.7 ± 7.3a	94.3 ± 2.3ab
Valmaine	95.0 ± 2.2a	86.7 ± 3.3a	48.5 ± 4.5a	96.6 ± 3.0a

For each cultivar, $n = 6$. For each cultivar, 60 alates in total were tested on six plants. Survival data were transformed by arcsin$\sqrt{x}$, and the numbers of offspring were transformed by $\sqrt{x}$ before analysis of variance. Values in each column followed by different letters were significantly different (Tukey–Kramer multiple range test; $P \leq 0.05$ [SAS Institute 2002]).

Host Preference and Reproduction of Alates in Choice Bioassay. *N. ribisnigri* alates showed considerable movement among lettuce plants and preference for certain susceptible cultivars over time (Table 3). One day after alate release, there were no significant differences in the number of alates on lettuce plants among the nine lettuce cultivars, regardless whether plants express resistance to *N. ribisnigri* ($F = 2.06$; df = 8, 43; $P = 0.062$), indicating random landing of alates on lettuce plants. From day 2 on, there were significant differences among lettuce cultivars in the number of alates per plant ($F = 11.96$; df = 8, 43; $P < 0.0001$ for day 2; $F = 15.73$; df = 8, 43; $P < 0.0001$ for day 3; $F = 17.71$; df = 8, 43; $P < 0.0001$ for day 4; $F = 15.08$; df = 8, 43; $P < 0.0001$ for day 5) (Table 3). The numbers of alates on plants of the two resistant cultivars declined dramatically by day 2 and 3 respectively. After day 3, the mean number of alates on a resistant plant was less than one, and the mean number of alates on a plant of susceptible cultivars ranged from four to 23. Generally, the numbers of alates on plants of resistant cultivars were significantly lower than those on plants of susceptible cultivars. The alate numbers on susceptible plants only showed small variations over time with the exception of Margarita, which had a steady increase in the number of alates over time, and the number was significantly higher than those on other susceptible plants (Table 3).

By day 5 after release of alates, the total numbers of offspring on lettuce plants also varied significantly among cultivars with significantly fewer offspring on resistant cultivars than on susceptible cultivars ($F = 15.59$; df = 8, 43; $P < 0.0001$) (Table 3). *N. ribisnigri* offspring also had significant lower survival rate on resistant cultivars than on susceptible cultivars ($F = 29.74$; df = 8, 43; $P < 0.0001$). Nymphs on resistant Dynamite and Barcelona had survival rates of 40.5 and 8.8%, respectively, compared with ≥89% on susceptible cultivars (Table 3). Across all cultivars, the mean of offspring on a plant of each cultivar was highly correlated with the 5-d average of alates per plant of each cultivar ($r^2_{adj} = 0.974$, $P < 0.0001$) (Fig. 1).

Survival and Population Growth on Susceptible and Resistant Cultivars in Separate and Mixed Treatments. The effects of plant type (with or without the resistance gene) were highly significant at both 14 and 21 d, indicating significant differences in aphid population size between susceptible and resistant cultivars (Table 4). There were no significant treatment effects, indicating that testing susceptible plants and resistant plants together (mixed treatment) or separately (separate treatment) did not have significant effects on population size. There were also no significant interactions between the treatment and plant type for the numbers of *N. ribisnigri* at 14 and 21 d (Table 4), indicating consistent effects of plant types

Table 3. Mean numbers ± SE of *N. ribisnigri* alates on nine lettuce cultivars 1 to 5 d after release and their reproduction and offspring survival at 5 d

Cultivar	No. alates/plant after alate release					Offspring/plant	Offspring survival (%)
	1 d	2 d	3 d	4 d	5 d		
Dark Green Boston	9.7 ± 2.8a	11.3 ± 3.3a	10.3 ± 2.8ab	10.3 ± 4.0b	11.8 ± 5.7b	94.7 ± 30.9ab	97.9 ± 1.0a
Dynamite	3.1 ± 1.5a	0.7 ± 0.4c	0.8 ± 0.8c	0.5 ± 0.3c	0.8 ± 0.5c	6.3 ± 2.5c	40.5 ± 11.7b
Margarita	7.8 ± 2.1a	13.5 ± 1.6a	17.5 ± 2.5a	19.7 ± 2.4a	23.0 ± 2.9a	156.7 ± 23.2a	99.7 ± 0.3a
Barcelona	6.2 ± 2.2a	2.0 ± 1.0bc	0.7 ± 0.3c	0.7 ± 0.5c	0.7 ± 0.2c	4.5 ± 2.3c	8.8 ± 6.1c
Calmar	9.7 ± 1.7a	10.7 ± 2.5a	10.5 ± 2.2ab	10.7 ± 1.6ab	8.3 ± 0.7b	88.2 ± 18.7ab	89.0 ± 6.9a
Salinas	5.8 ± 2.7a	7.2 ± 3.0ab	8.5 ± 3.9b	6.3 ± 3.3b	5.5 ± 1.5b	75.5 ± 42.5b	92.6 ± 4.0a
Lobjoits	6.0 ± 1.7a	7.7 ± 2.2a	8.7 ± 2.4ab	6.5 ± 1.8b	8.8 ± 2.8b	74.3 ± 20.9ab	99.8 ± 0.2a
Parris Island	10.2 ± 2.3a	11.0 ± 2.0a	10.3 ± 2.0ab	9.0 ± 1.9b	11.0 ± 3.1b	99.2 ± 21.5ab	95.6 ± 1.8a
Valmaine	8.5 ± 2.5a	7.5 ± 1.7a	6.0 ± 1.8b	4.7 ± 1.2b	4.2 ± 1.0bc	52.2 ± 16.6b	97.8 ± 1.2a

Survival data were transformed by arcsin$\sqrt{x}$, and the numbers of offspring were transformed by $\sqrt{x}$ before analysis of variance. Values in each column followed by different letters were significantly different (Tukey–Kramer multiple range test; $P \leq 0.05$ [SAS Institute 2002]).

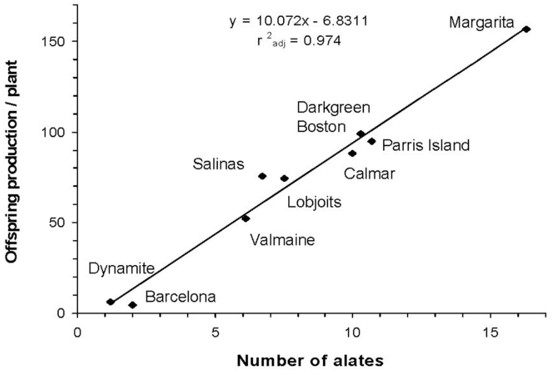

Fig. 1. Regression between the mean number of alates per plant and their offspring production for each cultivar 5 d postinfestation.

in both separate and mixed treatments. The significant cultivar effects nested in plant type for colony size at 21 d suggested significant differences among cultivars within plant type (Table 4).

In both separate and mixed treatments, *N. ribisnigri* successfully colonized plants from susceptible cultivars and failed to colonize plants from resistant lines (Table 5). Comparing treatment and plant type combinations, the numbers of aphid on resistant plants were significantly lower than those on susceptible plants in both treatments (Table 5). No live aphids were found on any of the resistant plants at 14 and 21 d in the separate treatment and an average of less than one aphid was found on a resistant plant in the mixed treatment. In comparison, the mean numbers of aphids on a susceptible plant were ≥12 at 14 d and ≥31 at 21 d (Table 5).

Within each treatment, the mean numbers of *N. ribisnigri* varied significantly among cultivars (Table 6). In the separate treatment, *N. ribisnigri* colonized all susceptible cultivars but did not survive on resistant cultivars. Population sizes on susceptible cultivars ranged from 3.2 to 26.0 at 14 d and from 11.6 to 76.8 at 21 d. In the mixed treatment, *N. ribisnigri* population sizes ranged from 2.7 to 39.3 at 14 d and from 11.3 to 52.0 at 21 d on susceptible cultivars. The number of *N. ribisnigri* on resistant plants ranged from 0 to 1 at 14 d and from 0 to 1.7 at 21 d. Multiple range comparisons showed separations of some resistant cultivars from susceptible cultivars and overlaps in others because of large variation of the data (Table 6).

Table 4. **Analysis of variance on *N. ribisnigri* colony sizes on resistant and susceptible lettuce plants in the separate and mixed treatments at 14 and 21 d**

Time	Source	df	F	P
14 d	Treatment	1	6.67	0.0108
	Plant type	1	199.47	<0.0001
	Cultivar (plant type)	19	1.77	0.0317
	Treatment × plant type	1	1.22	0.2713
	Error	139		
21 d	Treatment	1	0.03	0.8560
	Plant type	1	297.60	<0.0001
	Cultivar (plant type)	19	2.33	0.0028
	Treatment × plant type	1	1.69	0.1958
	Error	139		

Aphid counts were transformed by $\sqrt{x}$ before analysis of variance (SAS Institute 2002).

Table 5. **Mean ± SE colony sizes of *N. ribisnigri* on susceptible and resistant lettuce plants in separate and mixed treatments at 14 and 21 d**

Treatment	Plant type	n	No. of aphids/plant	
			14 d	21 d
Separate	Susceptible	65	12.1 ± 1.5b	39.0 ± 4.2a
	Resistant	40	0c	0b
Mixed	Susceptible	36	19.1 ± 2.8a	31.6 ± 3.3a
	Resistant	24	0.4 ± 0.1c	0.7 ± 0.2b

Numbers of aphids were transformed by $\sqrt{x}$ before analysis of variance. Values in each column followed by different letters were significantly different (Tukey–Kramer multiple range test; $P \leq 0.05$ [SAS Institute 2002]).

Survival and Reproduction of Alates on Leaf Discs. Survival of alates on leaf discs did not differ significantly between susceptible cultivars and resistant lines at 3 d ($F = 0.29$; df = 1, 46; $P = 0.595$). Numbers of offspring and survival rate of offspring on susceptible plants were significantly higher than those on resistant plants ($F = 12.71$; df = 1, 46; $P = 0.0009$ for offspring production; $F = 11.06$; df = 1, 46; $P = 0.0017$ for offspring survival rate) (Table 7). There were no significant interactions between plant type and cultivars, indicating all cultivars within each plant type had similar effects on the offspring production of *N. ribisnigri* alates and offspring survival.

Survival and Reproduction of Alates in Leaf Cages. In the clip-on leaf cage bioassays, *N. ribisnigri* alates did not survive to 7 d on leaves of resistant 85-45-RZ and did not produce offspring. On the susceptible Oakleaf, *N. ribisnigri* alates had a survival rate of 35.0 ± 10.9% and produced 3.7 ± 1.0 offspring in a leaf cage on average, values that were significantly higher than those on the resistant line ($F = 10.23$; df = 1, 38; $P = 0.0028$ and $F = 21.56$; df = 1, 38; $P < 0.0001$ for alate survival and offspring production, respectively). The results indicated that leaf cage bioassays of 7 d can separate resistant plants from susceptible plants effectively for resistance screening.

Discussion

Consistent results of high survival and reproduction of *N. ribisnigri* on most lettuce cultivars that do not express the resistance gene *Nr* highlight the vulnerability of most commercial lettuce cultivars to infestation by this pest and the importance of *N. ribisnigri* to lettuce production. The high resistance levels expressed by various breeding lines used in this study suggest that breeding lettuce plants expressing the *Nr* gene may be an effective tool to manage *N. ribisnigri.* The successful separation between resistant lines and susceptible cultivars in various bioassays using whole plants or leaf cages offered several options for resistance screening in lettuce breeding. Leaf disc bioas-

Table 6. Mean ± SE colony sized of *N. ribisnigri* on individual plants of susceptible and resistant cultivars in the separate and mixed treatments at 14 and 21 d

Cultivar	Plant type	Separate treatment		Mixed treatment	
		14 d	21 d	14 d	21 d
Bibb	S	10.6 ± 3.1ab	45.6 ± 12.6ab	9.0 ± 4.0abc	29.0 ± 13.5abc
Big Red	S	17.8 ± 6.7ab	43.2 ± 15.0ab	10.7 ± 3.7abc	40.3 ± 9.9a
Darkland	S	8.2 ± 4.9abc	19.8 ± 6.4abc	20.3 ± 11.3abc	31.3 ± 16.4abc
Lollo Rosa	S	15.6 ± 2.8ab	52.6 ± 13.6ab	22.3 ± 1.7ab	38.0 ± 11.0a
Margarita	S	26.0 ± 7.3a	76.8 ± 14.2a	17.7 ± 7.2abc	39.7 ± 12.7a
Pacific	S	20.4 ± 9.6ab	52.8 ± 16.5ab	13.7 ± 11.2abc	25.3 ± 7.2abcd
Parris Island	S	3.2 ± 1.4bc	12.2 ± 5.3bc	2.7 ± 1.5bc	11.3 ± 4.2abcde
Prizehead	S	4.6 ± 1.9bc	11.6 ± 3.0bc	26.3 ± 21.8abc	18.0 ± 2.0abcde
Salinas	S	6.2 ± 3.4bc	34.8 ± 13.3ab	25.3 ± 11.3ab	28.7 ± 17.3abcd
Shining Star	S	12.6 ± 3.6ab	34.0 ± 9.4ab	15.3 ± 4.8abc	27.3 ± 3.3ab
Tiber	S	14.2 ± 5.5ab	37.0 ± 17.3ab	39.3 ± 4.3a	40.0 ± 15.0a
Two Star	S	8.6 ± 6.2abc	46.2 ± 27.4ab	26.7 ± 11.3ab	52.0 ± 11.0a
Vanguard 75	S	9.0 ± 3.2abc	39.6 ± 14.7ab		
83-67RZ	R	0c	0c	0.3 ± 0.3bc	0.7 ± 0.7de
Campionas	R	0c	0c	0.7 ± 0.3bc	0e
Dynamite	R	0c	0c	0c	0e
Elenas	R	0c	0c	1.0 ± 0.6bc	0.3 ± 0.3e
Fortunas	R	0c	0c	0c	0.7 ± 0.3de
Irina	R	0c	0c	0.3 ± 0.3bc	1.0 ± 0.6bcde
Krinas	R	0c	0c	0.3 ± 0.3bc	1.7 ± 1.7cde
Vetonas	R	0c	0c	0c	0.7 ± 0.7de

S and R denote for susceptible and resistant plant types, respectively. Data were transformed by $\sqrt{x}$ before analysis of variance. Values in each column followed by different letters were significantly different (Tukey–Kramer multiple range test; $P \leq 0.05$ [SAS Institute 2002].

says, however, were not suitable for resistance screening.

Both nymphs and alates can be used to screen for resistance because susceptible and resistant plants were successfully separated based on 3- and 5-d survival for both nymphs and alates. Survival rates of nymphs and alates were also not significantly different on either susceptible plants or resistant plants. This also suggests that both stages were equally susceptible to the resistance gene *Nr.* The effects of resistant lettuce lines on *N. ribisnigri* were consistent with previous results of biotype nonspecificity of resistance (Eenink and Dieleman 1982). Greater separation between resistant and susceptible plants was obtained with the 5-d bioassay than the 3-d bioassay. Considering the lower proportion of alates in *N. ribisnigri* colonies and flight ability, screening by using nymphs may be preferred because of their availability and ease of handling.

Alates tested in cages on individual plants and alates released over a mix of resistant and susceptible plants represented no-choice and choice situations. In both types of tests, resistant plants and susceptible plants were clearly separated on the basis of alate survival and offspring production. In the choice tests (Table 3), alate distributions shifted from an initial random distribution to concentration on susceptible plants. Because the choice tests resulted in the same power of separation between resistant and susceptible plants, choice tests can be a simpler screening method for breeding lettuce resistant to *N. ribisnigri* by using the *Nr* gene. The strong correlation between number of alates and number of offspring on susceptible and resistant cultivars (Fig. 1) suggests that reproduction per alate remained same regardless of plant cultivar.

In comparison with low survival rates on resistant plants at 3 and 5 d in bioassays with nymphs and alates, no *N. ribisnigri* survived in 7-d leaf cage bioassays or at 14 d in the separate treatment. The survival in the short-term tests indicates slow effects of resistant plants, consistent with the primary mode of action of feeding deterrence. In the mixed treatment, the low numbers of aphids on resistant plants at 15 and 21 d were likely because of random dispersal of alates to resistant plants and offspring production by those alates. Small nymphs were used in the 14 and 21 d mixed treatments, and they became mature before 14 d when numbers of *N. ribisnigri* on lettuce plants were recorded.

There was no significant difference between resistant and susceptible plants in alate survival in leaf disc bioassays. The low survival rate of ≈30% on discs of susceptible leaves is indicative of the difficulty in maintaining optimal conditions in the leaf disc bioassay. The differences in offspring production and offspring survival between resistant and susceptible

Table 7. Mean ± SE survival, offspring production, and offspring survival of *N. ribisnigri* alates on leaf discs from lettuce plants of 14 susceptible cultivars and 10 resistant cultivars at 3 d

Plant type	*n*	Alate survival (%)	Offspring	Offspring survival (%)
Susceptible	28	30.0 ± 5.1a	16.9 ± 1.6a	77.6 ± 3.3a
Resistant	20	24.0 ± 4.9a	9.1 ± 1.5b	51.7 ± 7.7b

Survival data were transformed by arcsin$\sqrt{x}$, and the numbers of offspring were transformed by $\sqrt{x}$ before analysis of variance. The two values in each column followed by different letters were significantly different based on significant plant type effect ($P \leq 0.05$; [SAS Institute 2002]).

plants were also much smaller in leaf disc bioassays than in whole plant bioassays. This suggests that resistance in leaf discs may decline over time. This potential concern as well as the difficulty to maintain leaf discs or detached leaves in turgid state for prolonged period make leaf discs bioassay unsuitable for resistance screen in lettuce breeding. In contrast, leaf cage bioassays seem to be an effective screening method based on successful separation of resistant plants and susceptible plants and no survival on resistant plants in 7 d. This method may be particularly useful in conducting screening in the field where release of *N. ribisnigri* in open field is a concern and natural infestation is not reliable or adequate for screening purpose.

In summary, resistant and susceptible plants can be separated in 3-d whole plant bioassays. Longer term bioassays were more powerful. Either nymphs or alates were equally suitable for resistance screening. Plants can be inoculated individually with *N. ribisnigri* nymphs or alates. Alternatively, *N. ribisnigri* alates can be released to a mix of resistant and susceptible plants for resistance screening. Leaf cage bioassay was also an effective screen method. Leaf disc bioassay is not suitable for resistance screening. All lettuce cultivars without resistance gene *Nr* were susceptible to *N. ribisnigri,* indicating the importance of this pest to lettuce production. The effective control of *N. ribisnigri* on lettuce plants with *Nr* gene shows good potential of *Nr* gene to be used in breeding resistant lettuce cultivars.

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